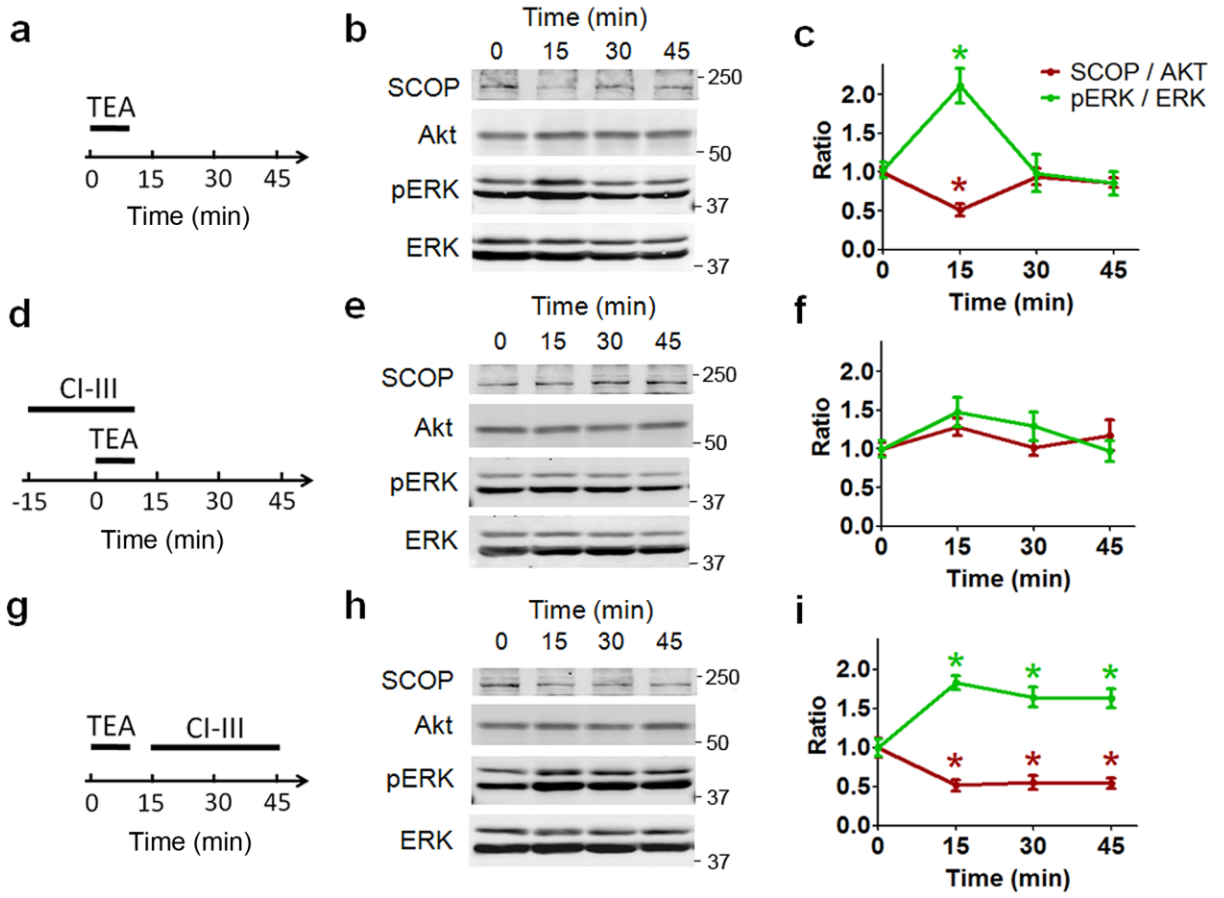
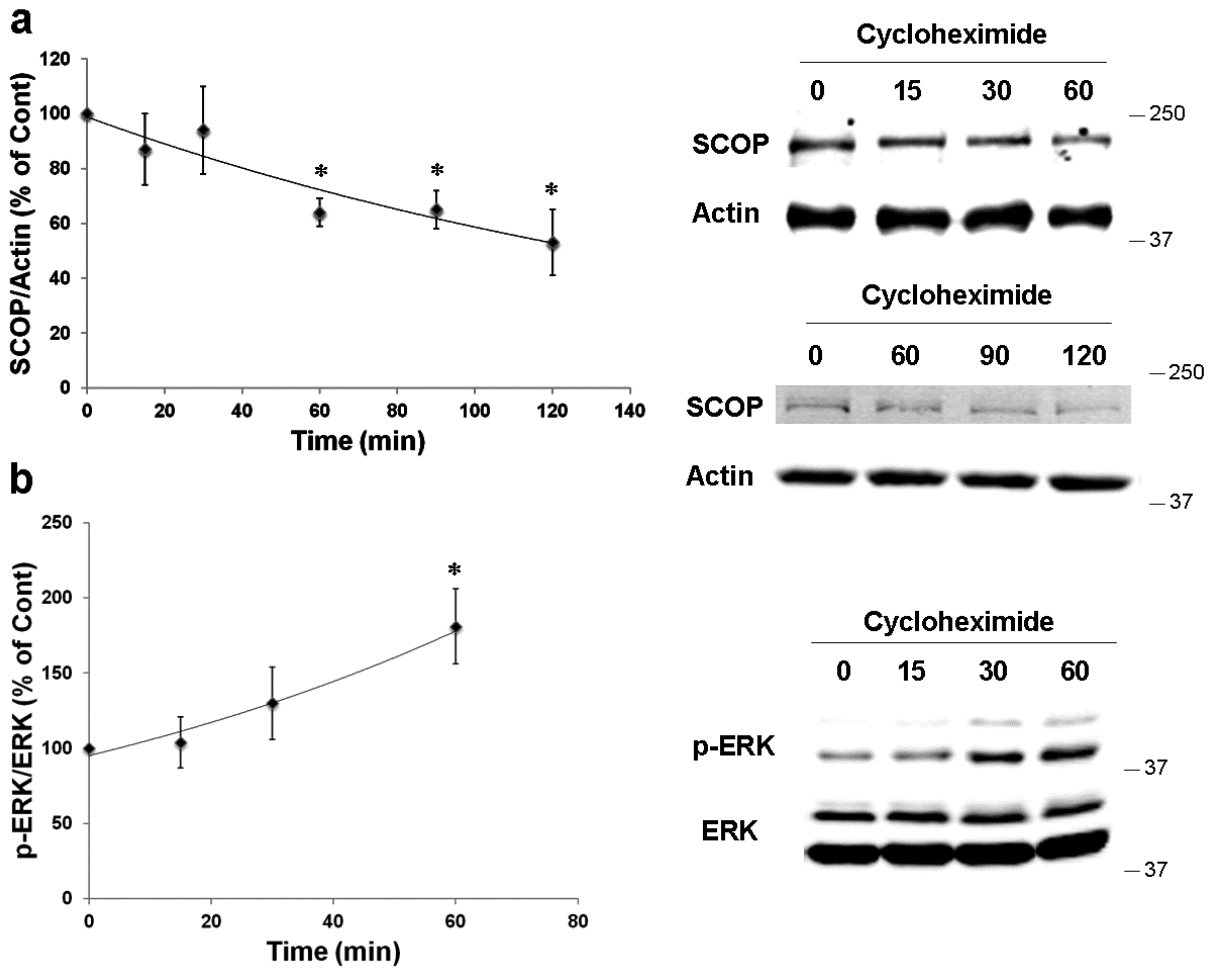
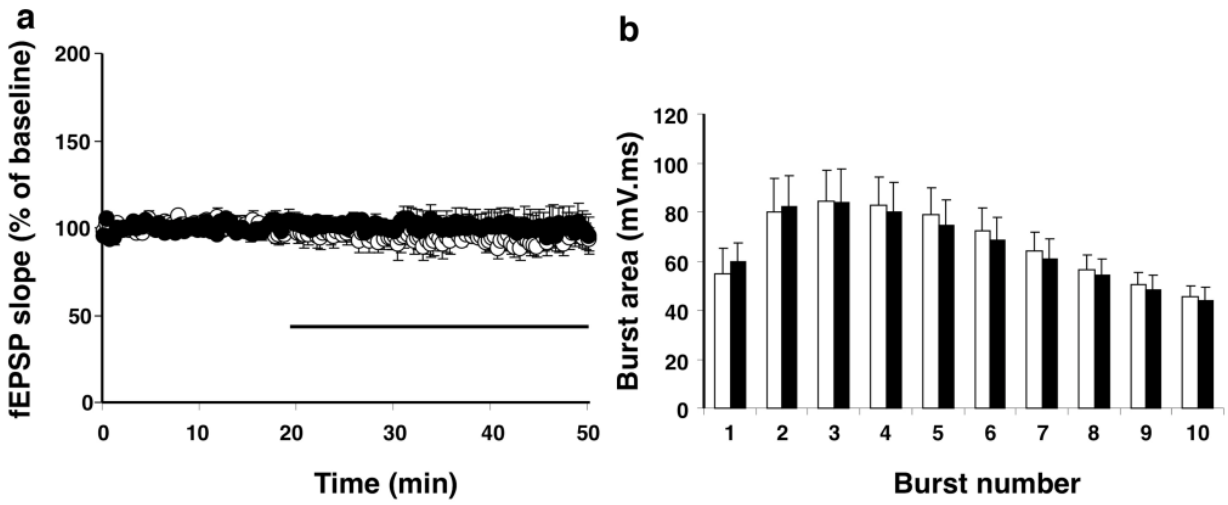


Supplementary Figure 1: Effects of calpain inhibition on TEA-induced changes in SCOP and p-ERK in adult hippocampal slices.

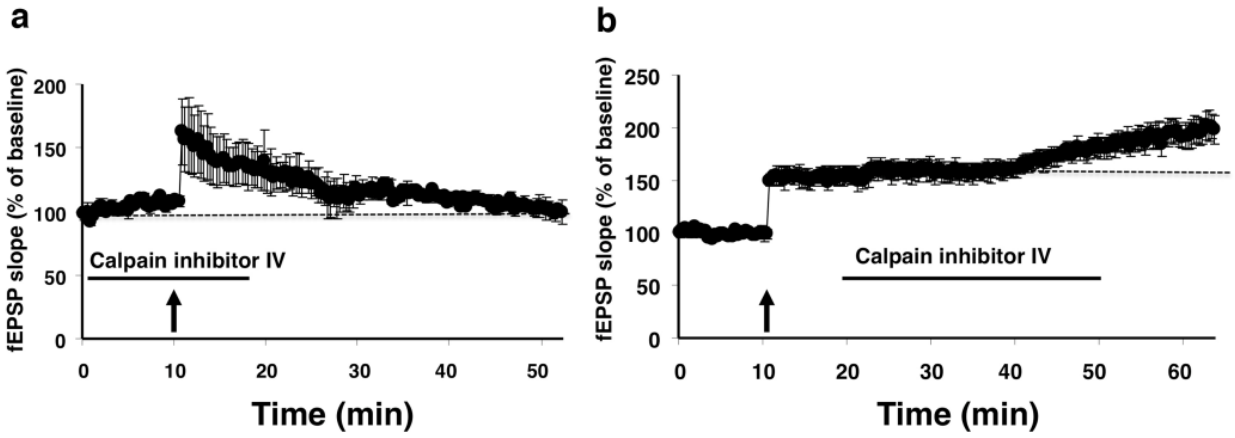
a, d, g. Experimental protocols: hippocampal slices were prepared from adult rats and were treated with TEA (20 mM) for 10 min in the absence or presence of calpain inhibitor III (10 µM), as indicated. **b, e, h.** Representative western blots for SCOP, Akt, p-ERK and ERK under various experimental conditions. **c, f, i.** Quantitative analysis of the levels of SCOP (normalized to the values of Akt) and p-ERK/ERK ratios under various experimental conditions. In all cases, results are means ± S.E.M. of 3 experiments. * $p < 0.05$, as compared to time point 0, one-way ANOVA followed by Bonferroni test.



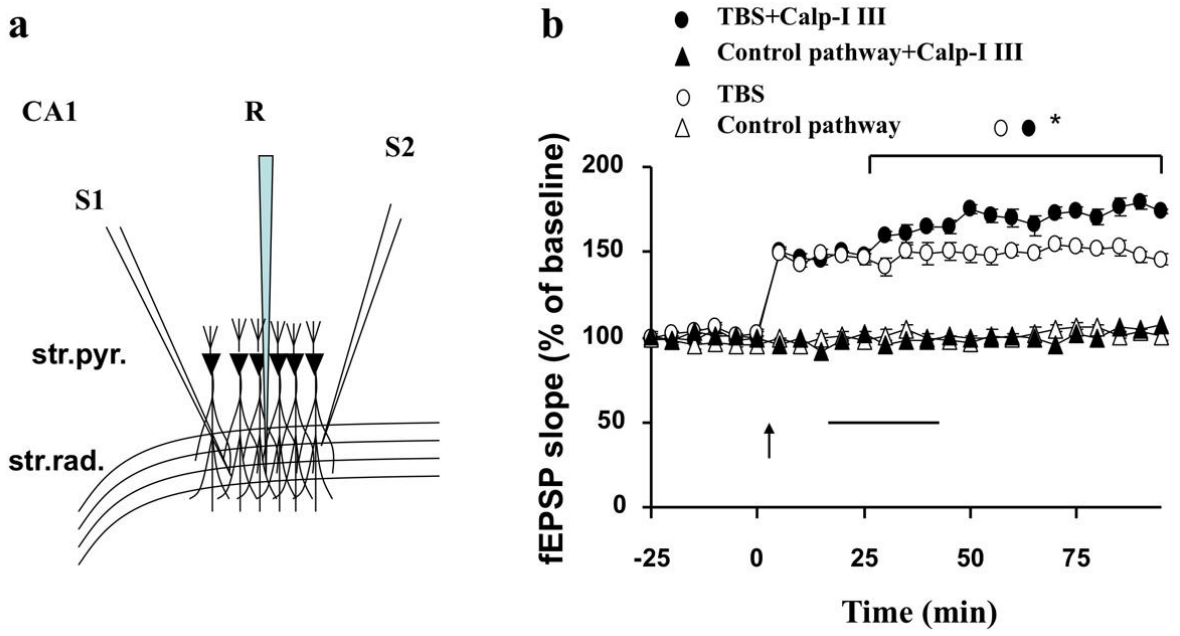
Supplementary Figure 2: Effects of cycloheximide treatment of cortical synaptoneurosomes on SCOP and p-ERK levels. Cortical synaptoneurosomes were treated with cycloheximide (25 μM) for the indicated periods of time. Levels of SCOP, actin, p-ERK and ERK were determined with western blots. Results are means ± S.E.M. of 4-6 experiments. * $p < 0.05$, as compared to time point 0, one-way ANOVA followed by Bonferroni test. Note that the half-life for SCOP was determined to be about 90 min by assuming a single first-order reaction kinetics.



Supplementary Figure 3: Calpain inhibitor III has no effect on basal synaptic transmission or on the responses to theta burst stimulation. The values represent means ± S.E.M of 6-8 slices from 3-4 animals. No statistically significant differences were observed between vehicle-treated slices (open circles or bars) and calpain inhibitor III-treated slices (10 μM closed circles or bars) (two-tail Student's t-test).

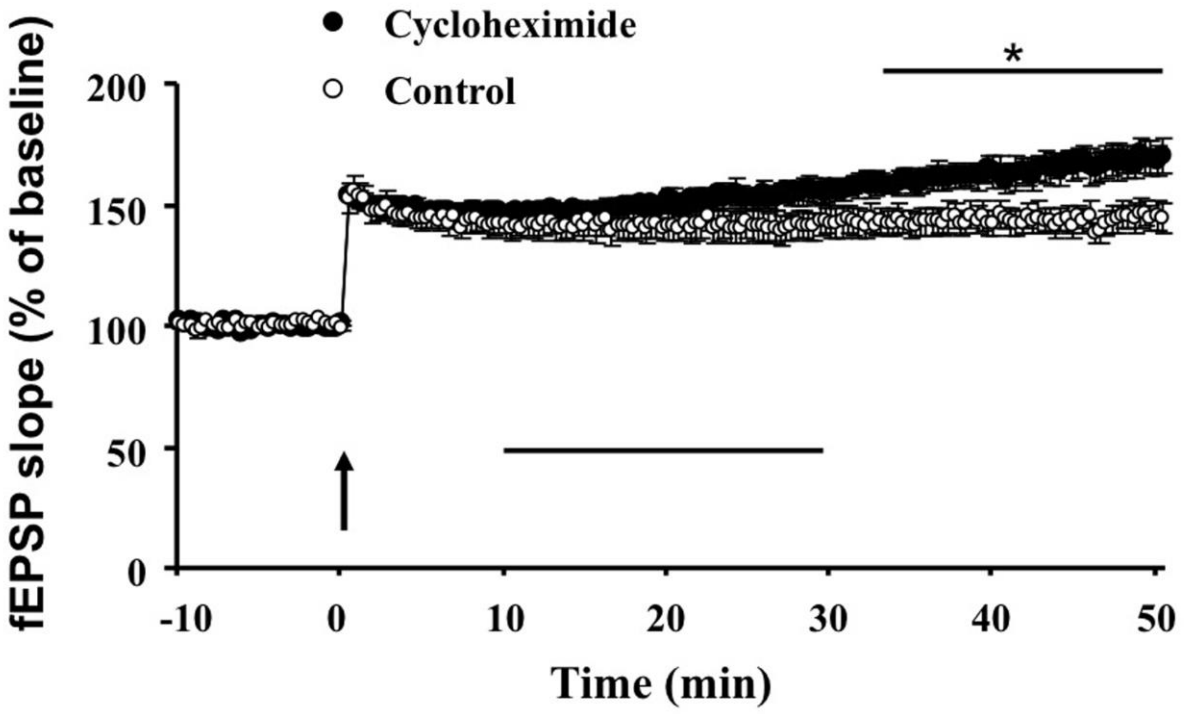


Supplementary Figure 4: Calpain inhibitor IV has the same effects on TBS-induced LTP as calpain inhibitor III, when applied before or after TBS. Calpain inhibitor IV (arrows, 2 μM) has a different profile for cysteine protease inhibition than calpain inhibitor III (see reference 19 in text of the manuscript). The slopes of fEPSPs are expressed as percent of the average values recorded during the 10 min baseline, and are means ± S.E.M. of 5-10 slices prepared from 3-5 animals.



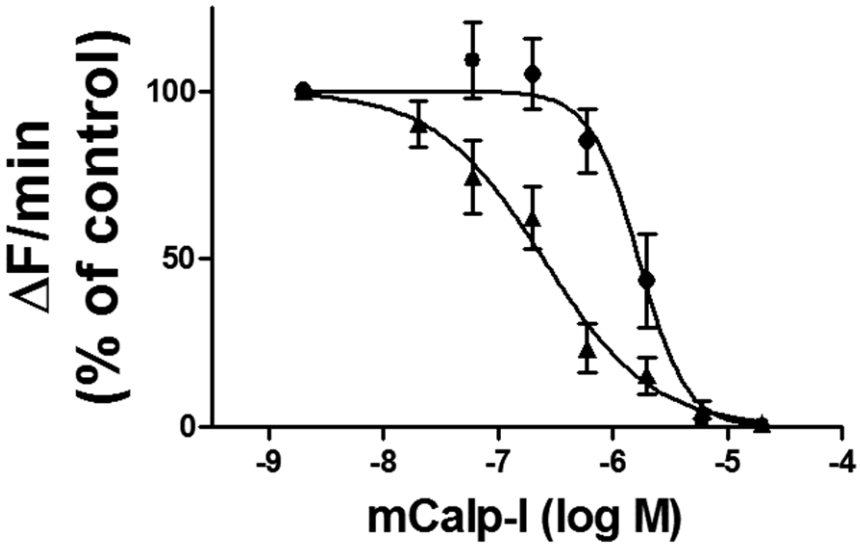
Supplementary Figure S5: Synapse specificity of calpain inhibitor-induced LTP enhancement.

a) Schematic representation of the relative positions of recording and stimulation electrodes in stratum radiatum (str. rad.) of hippocampal CA1 area. **b)** Calpain inhibitor III applied 10 min post TBS (horizontal line) enhanced LTP in S1 pathway (closed circles) as compared to vehicle-treated slices (open circles); from time 35 min and until the end of the recording, the differences were statistically significant (* $p < 0.01$; two-tail Student's t-test). However, the control pathway remained stable in the absence (open triangles) or presence (closed triangles) of calpain inhibitor III. The slopes of fEPSPs are expressed as percent of the average values recorded during the 25 min baseline, and are means ± S.E.M. of 5-10 slices prepared from 3-5 animals.



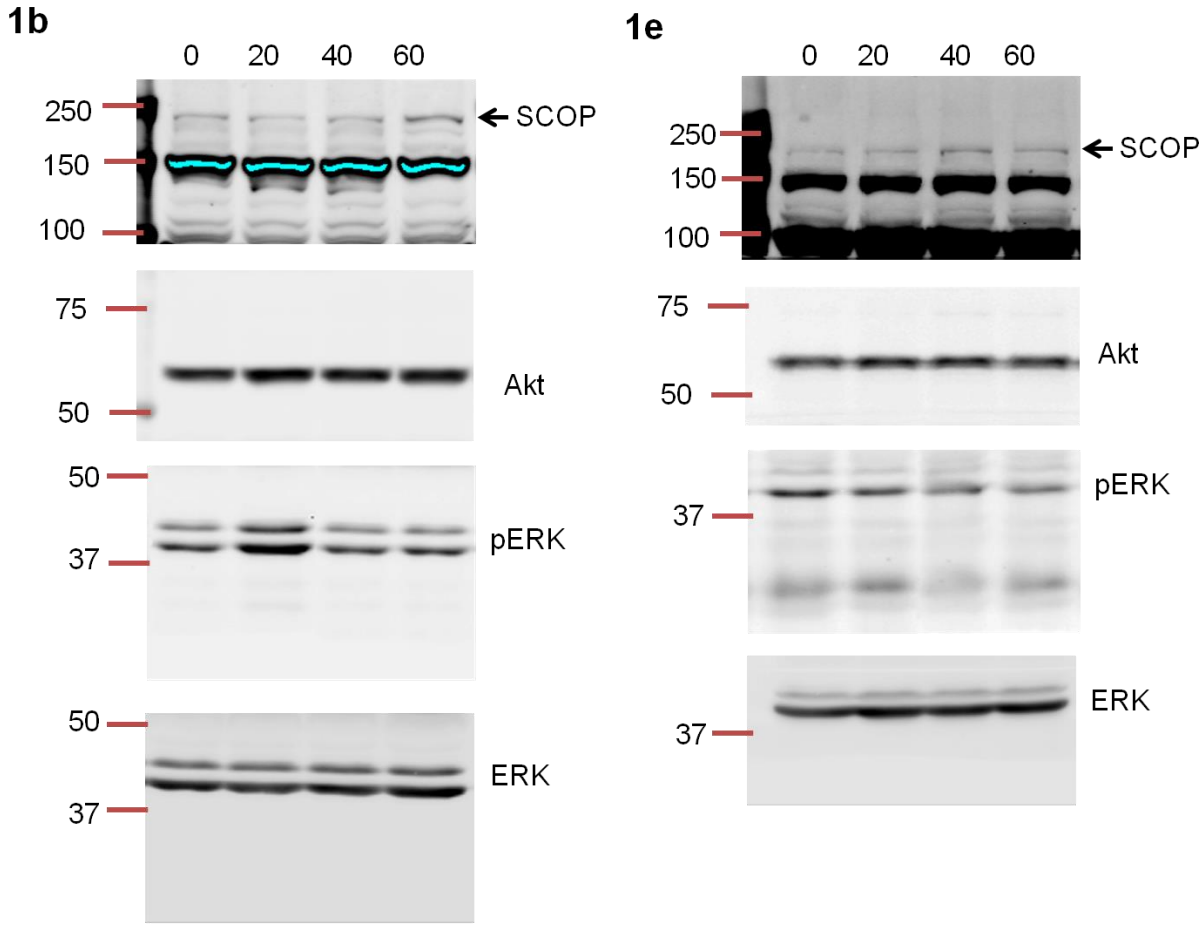
Supplementary Figure 6: Cycloheximide mimics calpain inhibition-induced LTP enhancement.

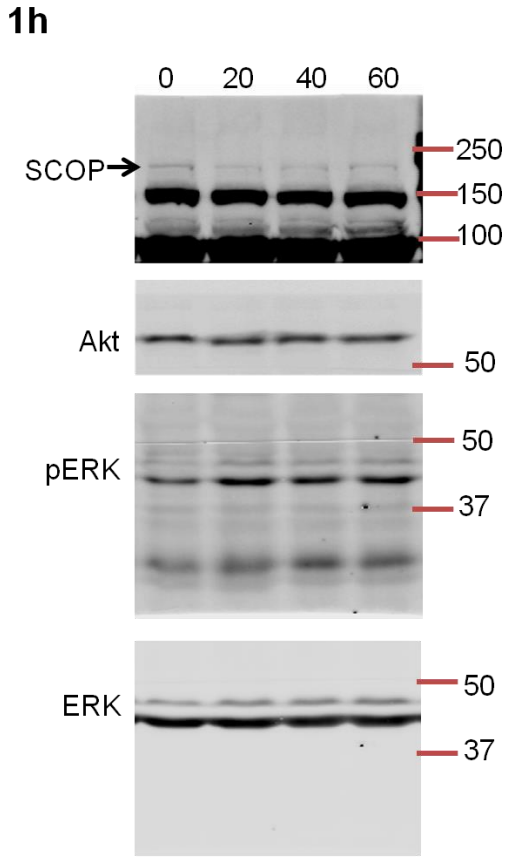
Cycloheximide (25 µM) was applied for 20 min (horizontal line) starting 10 min after TBS. The slopes of the fEPSPs are expressed as percent of the average values recorded during the 10 min baseline, and are means ± S.E.M. of 5-10 slices prepared from 3-5 animals. From time 35 min and until the end of the recordings, the differences between values in control slices (open circles) and cycloheximide-treated slices (closed circles) are statistically significant (* $p < 0.01$; two tail Student's t-test).

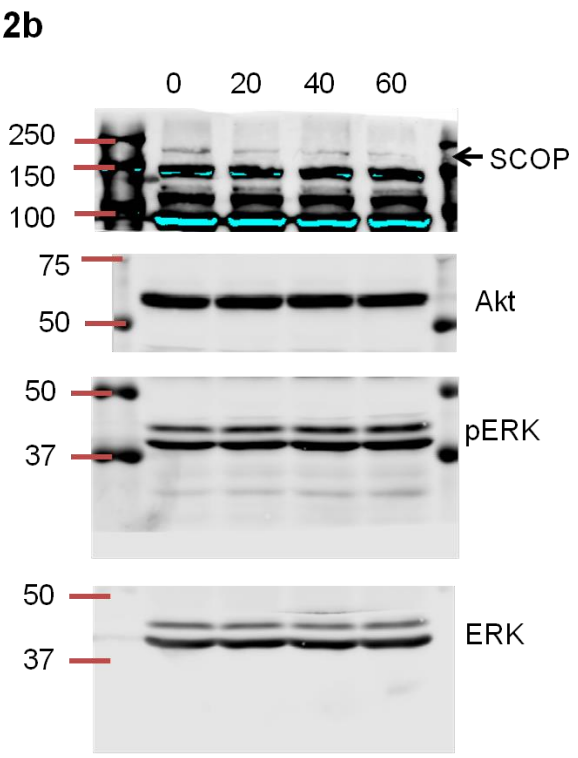


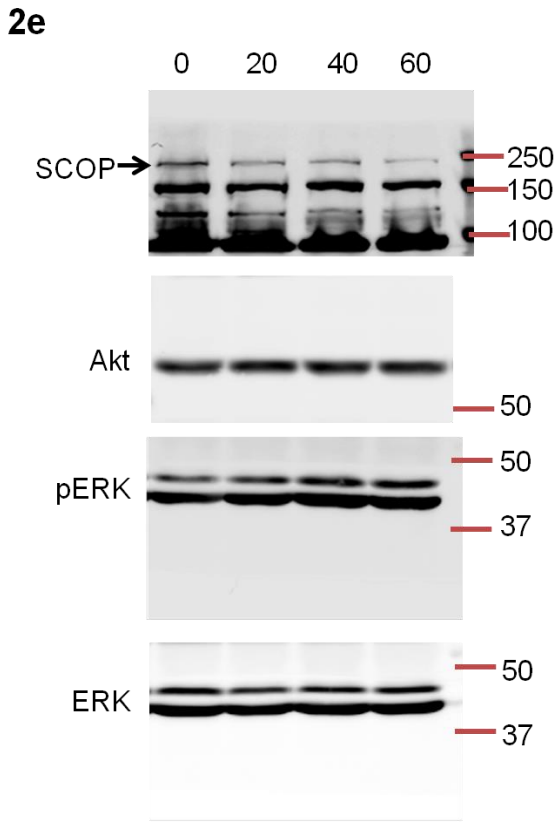
Supplementary Figure 7: Inhibition by mCalp-I of μ-calpain and m-calpain activity.

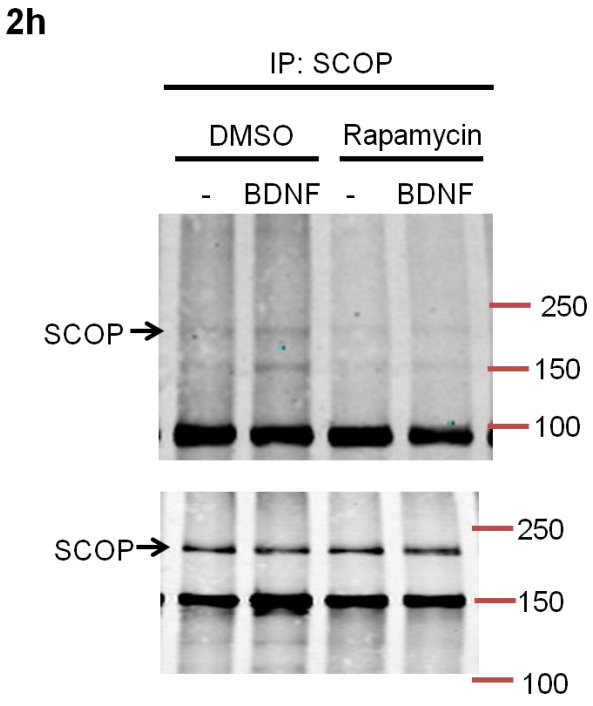
The fluorescence generated by Suc-Leu-Tyr-AMC cleavage was monitored after the addition of 8 μg of either μ-calpain (circles) or m-calpain (triangles) to a solution containing 0.5 mM of the fluorogenic substrate in the presence of different concentrations of mCalp-I (0-20 μM). Calpain activity was calculated as the slope of the changes of fluorescence as a function of time during the linear portion of the assay, and the results were expressed as percent of control (assay run in the absence of mCalp-I) and are means ± S.E.M of three independent experiments.

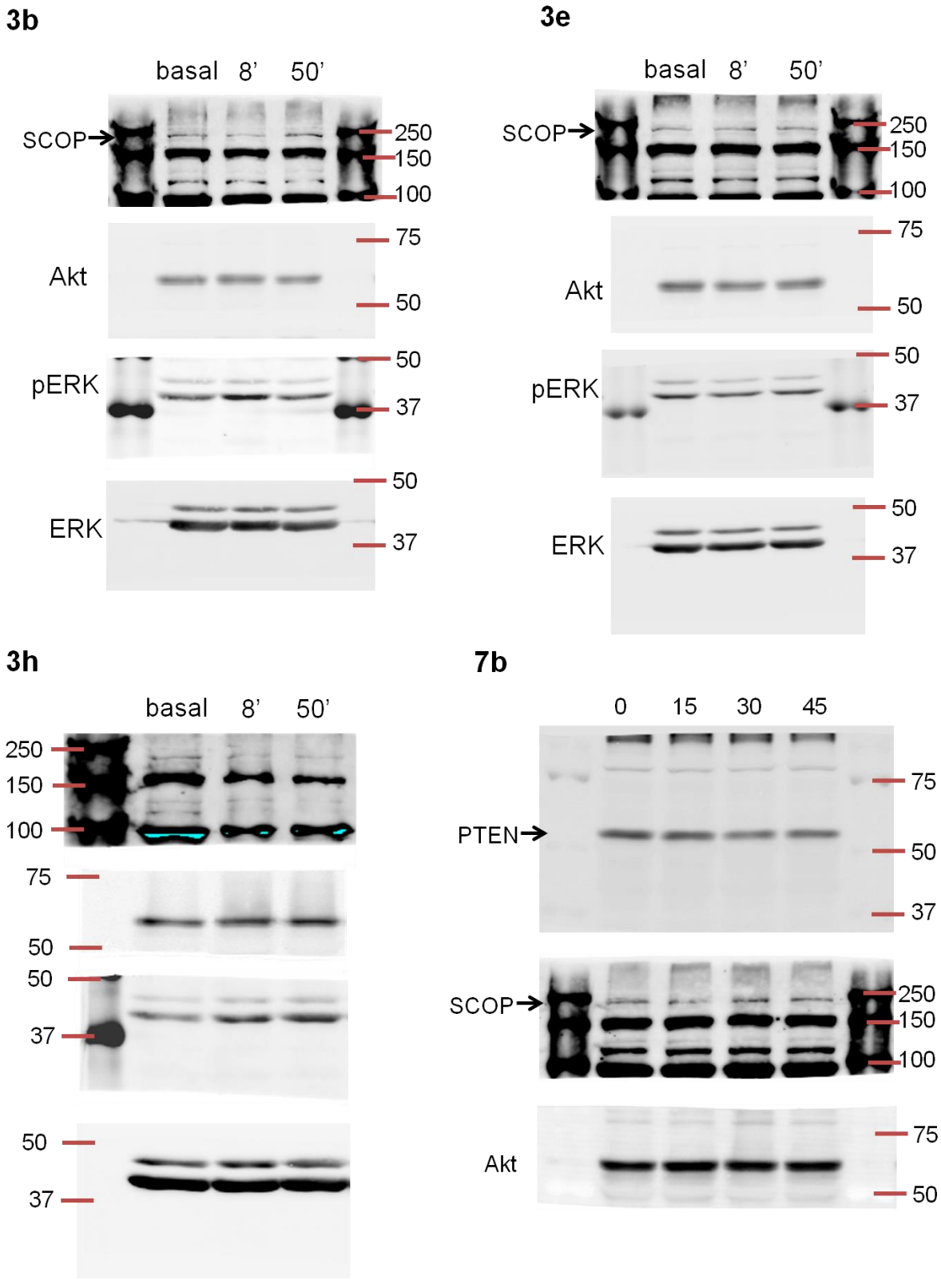

1b
0
20
40
60
250
150
100
SCOP
75
50
Akt
50
37
pERK
50
37
ERK
1e
0
20
40
60
250
150
100
SCOP
75
50
Akt
37
pERK
37
ERK


1h
0
20
40
60
SCOP
250
150
100
Akt
50
pERK
50
37
ERK
50
37

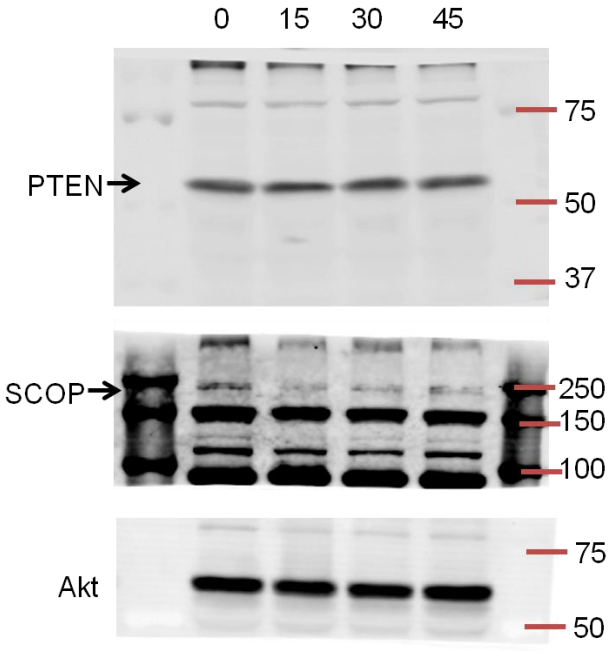

2b
0
20
40
60
250
150
100
SCOP
75
50
Akt
50
37
pERK
50
37
ERK


2e
0
20
40
60
SCOP
250
150
100
Akt
50
pERK
50
37
ERK
50
37

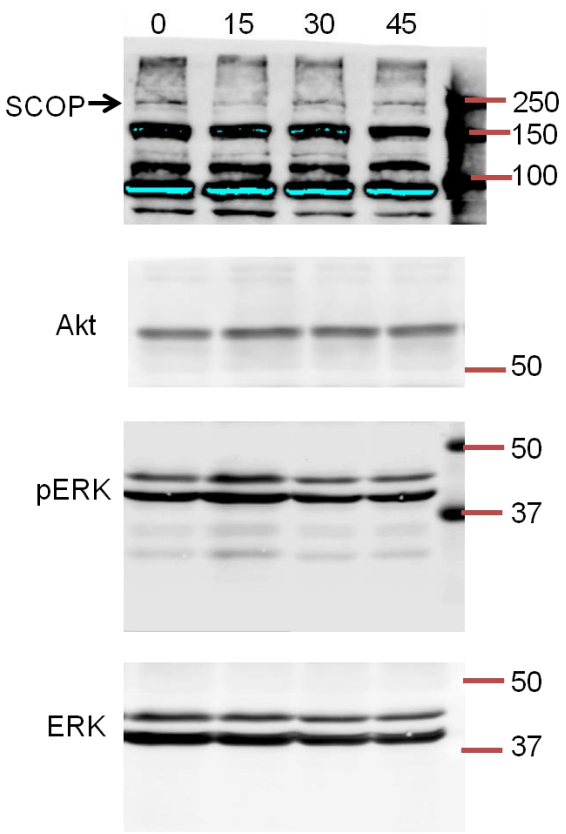

2h
IP: SCOP
DMSO
Rapamycin
-
BDNF
-
BDNF
SCOP
250
150
100
SCOP
250
150
100


3b
basal 8' 50'
SCOP
250
150
100
Akt
75
50
pERK
50
37
ERK
50
37
3e
basal 8' 50'
SCOP
250
150
100
Akt
75
50
pERK
50
37
ERK
50
37
3h
basal 8' 50'
250
150
100
75
50
50
37
50
37
7b
0 15 30 45
PTEN
75
50
37
SCOP
250
150
100
Akt
75
50

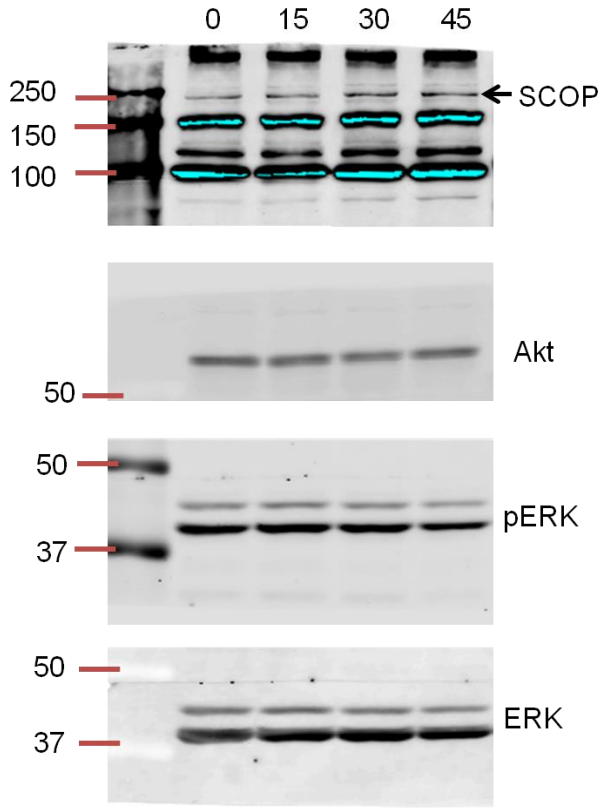
7e



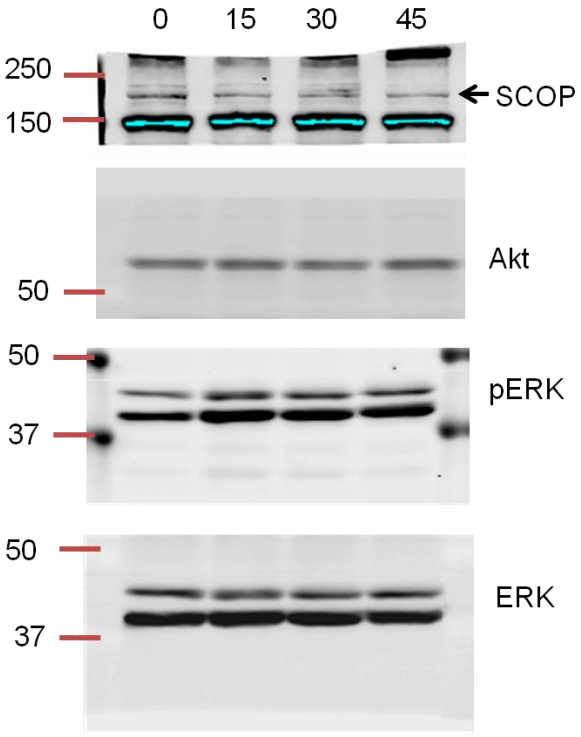
S1b

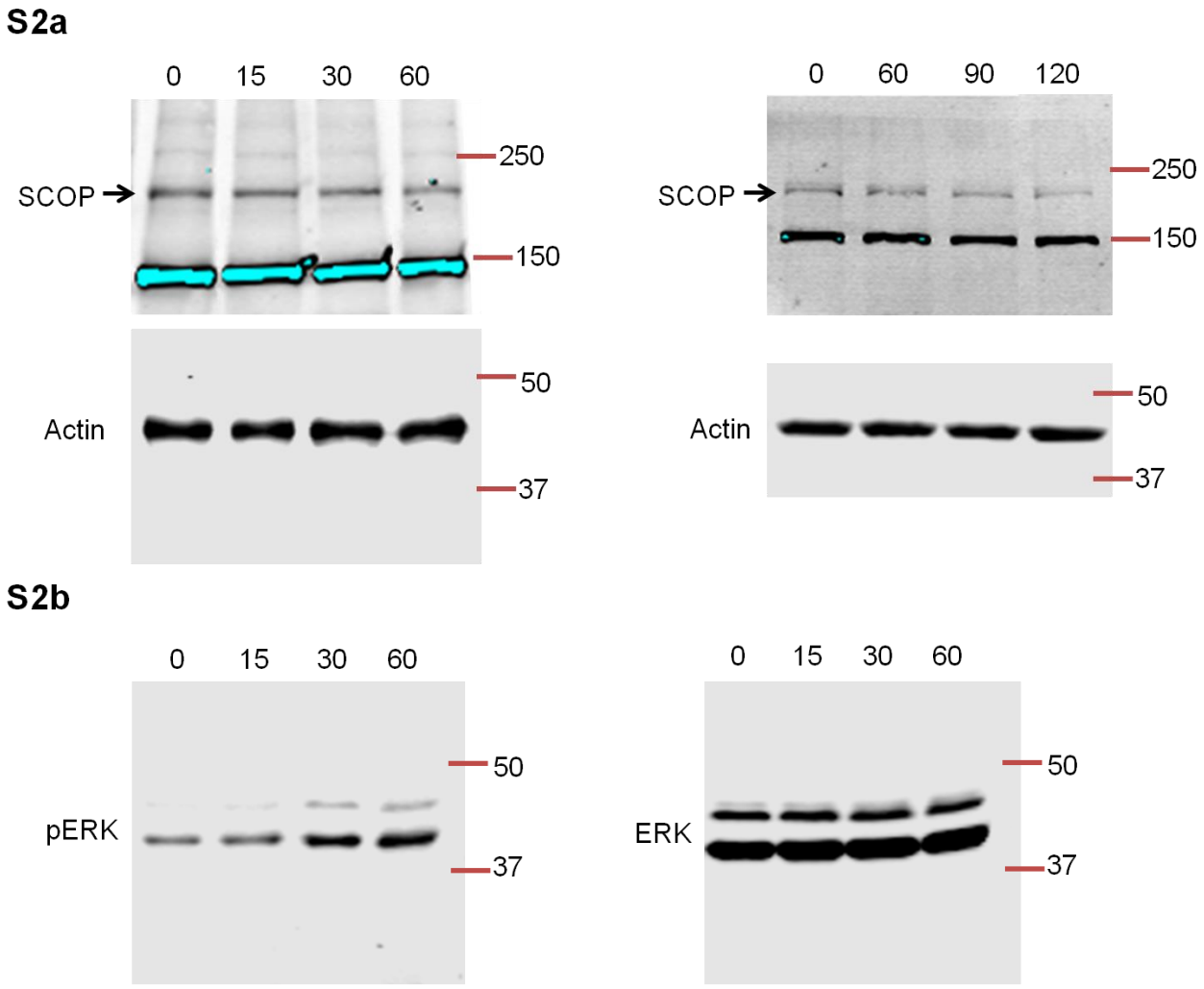


S1e



S1h





Supplementary Figure 8: Full gel scans of all western blots in article.
Each blot is labeled with its figure panel number.